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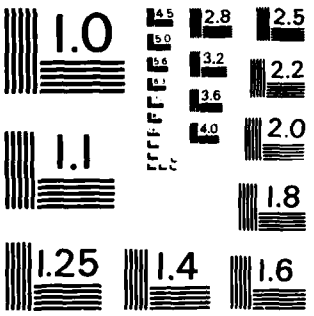

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ASPEN CENTER FOR PHYSICS
ASPEN, COLORADO

REPORT OF A WORKSHOP ON MEMBRANE BIOPHYSICS
HELD
JUNE 25 - JULY 15, 1984

SUPPORTED BY

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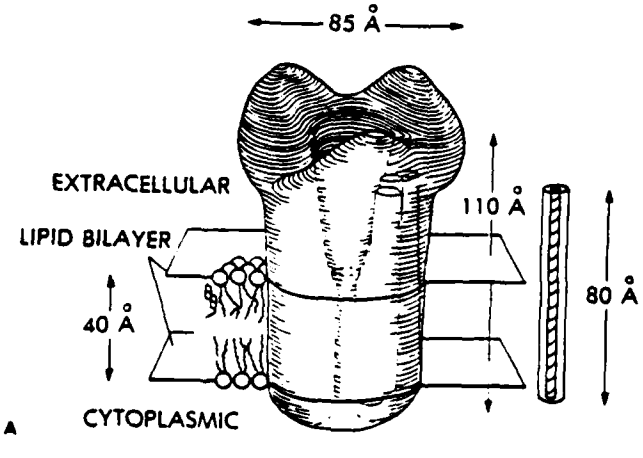

CONTENTS

Cover picture taken from:

Joerg Kistler, Robert M. Stroud, Michael W. Klymkowsky, Roger A. Lalancette, and Robert H. Fairclough, "Structure and Function of an Acetylcholine Receptor," Biophys. J. 37 (1982) pp. 371-383.

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WORKSHOP ON MEMBRANE BIOPHYSICS

Aspen Center for Physics

25 June - 13 July 1984

The idea behind this workshop, which was organized in collaboration with Lubert Stryer and Robert Knox, was to collect together a group of biologists and biophysicists with common interests in membrane biology. Our aim was to get a fairly diverse group to promote cross-fertilization of ideas among different specialties in membrane biophysics.

In the event, there were fourteen lectures spread over a period of three weeks from invitees, with an additional lecture on cell-cell contacts by Dr. George Bell (Los Alamos).

The abstracts for the invited lectures are included to form the report of the workshop. The subject matter may be classified as follows:

• Excitable membranes and voltage controlled gates	M. Blank and C. Stevens
• Photosynthetic reaction centers	S. Boxer, R. Knox and C. Swenberg
• Acetylcholine receptor	M. Montal, R. Stroud, and S. Doniach
• Visual transduction	L. Stryer
• Motility mechanism in bacteria	S. Block
• Protein-protein interactions	J. Owicki

The major emphasis of the Workshop was on person-to-person communication in addition to the more formal lectures. The Aspen Center for Physics provides a hospitable and stimulating environment for contacts and communication. We thank Sally Mencimer and her staff for the considerable amount of work that goes to provide the exceptional environment for scientific exchange.

We are grateful to Dr. Jeanine Majde, Office of Naval Research, for her generous support of the Workshop.

S. Doniach
Workshop Coordinator

31 October 84
SD/g

ELECTRICAL DOUBLE LAYERS IN ION TRANSPORT AND EXCITATION

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Our recent studies of electrical double layers at membrane surfaces have led to important new insights into membrane function. In particular, we have developed an explanation of the unusual ionic fluxes during excitation, consistent with the ideas of physical chemistry.

Interfacial effects are particularly important for ion transport processes in membranes, and we have developed a way of approximating the ionic processes in the electrical double layer region at charged surfaces using the Surface Compartment Model (SCM). The SCM treats the electrical double layers at the surfaces of natural membranes as compartments, and includes two surface potentials as well as two surface capacitances. It also considers ion concentration changes, and cation binding and release in the surface layers. The equations for this system are based on: 1) conservation of mass for ions, 2) conservation of surface charges, 3) conservation of charge during current flow across the different interfaces, and 4) the reaction kinetics of cation binding and release at the membrane surfaces. In the equations, the fluxes are given by Nernst-Planck expressions as in aqueous solution, where individual ion flows are proportional to the electrochemical potential differences. The equations for the system have been solved on a DEC minicomputer under several sets of conditions, using numerical values from studies of the squid axon and relevant physical models.

Using the SCM approximation for the electrical double layers of membranes, together with an equation for a channel where the cation permeability increases upon depolarization, we have obtained the membrane currents normally observed in voltage clamp experiments on squid axons, i.e., an initial inward current, followed by an outward current. The dependence of the current on clamp voltage, generally characterized in terms of the peak inward current and the steady state outward current, is similar to the curves published for squid axon.

Although any mechanism having a voltage dependent ion channel will give the above results with the SCM, we have developed a rationale for the opening of a channel during the electrical stimulation of excitable membranes, based on the dissociation of oligomeric proteins with increases in charge. The small and rapid shifts of charge called "gating currents" could cause the permeability changes if the channel is oligomeric, and dissociation is the molecular process that leads to opening. Because of the asymmetric charge distribution in the resting state, the channel is not uniformly associated. It is dissociated (i.e., open) on the outer surface, where the charge is high, and associated (i.e., closed) on the inner surface, where the charge is low.

Upon depolarization, the shift of negative charge from the outer to the inner surface causes the channel to open. Qualitatively, the properties of this voltage sensitive ion channel are compatible with observed membrane properties in terms of the steady state distribution of charge, the direction and magnitude of the shift of charge during depolarization, the range of surface charge where opening occurs, the cation selectivity of the channel, and the cation binding.

The introduction of surface compartments as approximations to electrical double layers has enabled us to gain insights into the role of ionic processes at membrane surfaces during transients. The SCM has also drawn attention to physical properties (e.g., surface capacitances) that were previously unnoticed and that may suggest mechanisms for the actions of different pharmacologic agents.

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ABSTRACT

SUCCESSIVE INCORPORATION OF FORCE-GENERATING UNITS IN THE BACTERIAL ROTARY MOTOR

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Mot mutants of *Escherichia coli* are paralyzed: their flagella appear to be intact but do not rotate. The *mot*A and *mot*B gene products are found in the cytoplasmic membrane; they do not co-purify with flagellar basal bodies isolated in neutral detergents. Silverman *et al.* found that *mot* mutants could be 'resurrected' through protein synthesis directed by λ transducing phages carrying the wild-type genes. Here, we have studied this activation at the level of a single flagellar motor. Cells of a *mot*B strain carrying plasmids in which transcription of the wild-type *mot*B gene was controlled by the *lac* promoter were tethered to a glass surface by a single flagellum. These cells began to spin within several minutes after the addition of a *lac* inducer, and their rotational speed changed in a series of equally spaced steps. As many as seven steps were seen in individual cells and, from the final speeds attained, as many as sixteen steips could be inferred. These experiments show that each flagellar motor contains several independent force-generating units comprised, at least in part, of *mot*B protein.

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ABSTRACT

THE MECHANISM OF CHARGE SEPARATION IN PHOTOSYNTHETIC REACTION CENTERS

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Photosynthetic reaction centers are the smallest unit of the photosynthetic membrane in which the initial photoinduced charge separation and stabilization characteristic of functional photosynthetic organisms takes place. The best characterized reaction centers from bacteria consist of three hydrophobic, transmembrane polypeptides, several reactive chromophores and a detergent used in the isolation. Extensive prior studies of the kinetics of charge separation show that the initial electron transfer reaction takes place within 1 psec following excitation. If other electron acceptors are removed, this initial charge separated species lives for 10-20 ns before recombinative decay to the ground or excited triplet state of the primary electron donor. Although the triplet state is not formed during the normal course of events, the mechanism of its formation has provided a great deal of insight into the nature of the initial charge separated state.

We have shown that the branching between ground and excited triplet state on charge recombination can be substantially affected by external magnetic fields. Such effects are observed because the initially formed radical ion pair is in a non-stationary state which can oscillate between singlet and triplet. The rate of this oscillation is directly affected by both internal (hyperfine) and external fields. Decay to the singlet ground or triplet excited state conserves angular momentum, consequently the rate of evolution of the spin multiplicity of the radical pair affects the yield of either product state. By analyzing the magnetic field dependence of the

triplet yield, the triplet lifetime, and the radical pair decay itself, it has been possible to obtain the following characteristics of the initial state: the unpaired spins in the pair interact very weakly by a through bond (electron exchange) mechanism; the unpaired spins interact relatively strongly through space (dipole-dipole) suggesting a separation of 7-8 Å between the donor and acceptor; the triplet decay rate constant is about one order of magnitude faster than the singlet decay rate constant, despite the fact that the driving force for the latter is much greater than the former -- this is suggestive of the inverted region predicted by Marcus theory; the radical pair energy lies about 0.2 eV below the energy of the excited singlet state, i.e. about 90% of the initial excitation energy is preserved in the primary charge separated state; the spin dynamics depends on orientation in a magnetic field, a new phenomenon which can be related to the orientations of the components in the reaction center and in the membrane; the expected magnetic isotope effects are not observed, but a novel two flash experiment causes transient formation of a non-equilibrium nuclear spin population distribution which can be related to the predicted magnetic isotope effect. Experiments are currently underway using single crystals of reaction centers which have recently become available.

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Abstract

LOCATION OF Tb^{3+} BINDING SITES IN ACETYLCHOLINE-RECEPTOR-ENRICHED MEMBRANES

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This is a report of research[1] conducted by Robert Fairclough, Richard Miake-Lye (Stanford University, Robert Stroud (University of California Medical School, San Francisco), Keith Hodgson and S. Doniach (Stanford University). In this research, the acetylcholine-receptor-enriched membrane from the electroplax of *torpedo californica* are treated with solutions of terbium chloride in the concentration range 20 to 50 μmolar. Titration studies show that the membrane binds roughly on the order of 46 Tb^{3+} ions per unit of receptor.

Small-angle x-ray diffraction on centrifuged pellets of the receptor preparations exploits the large energy-dependent changes in the Tb^{3+} x-ray scattering factor which occur near the L_{III} absorption edge at 7505 eV x-ray energy. Using known x-ray scattering phases[2] for the meridian (i.e., normal to the membrane), we can generate a difference Fourier map for the meridional diffraction amplitude at different x-ray energies below the edge and on the L_{III} absorption edge. At this stage the data very clearly reveals a set of well-localized binding regions for the cation. The subsequent iterative refinement step locates six regions of terbium binding across the membrane; most significant is the finding of substantial numbers of Tb^{3+} ions (21 and 8) near the entrance and exit of the ion channel, as well as of order three Tb^{3+} ions localized in the channel itself. This is the first physical evidence that cation-binding sites are to be found in the channel region of the receptor (inside the membrane bilayer) and strongly suggests the presence of anionic carboxylate side chains on the channel lining.[3]

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This work was supported by the National Institutes of Health grant GM25217.

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SD/g

ABSTRACT

Chlorophyll-protein absorption and fluorescence and exciton dynamics

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The structures of chlorophyll-protein (CP) complexes are well characterized by X-ray analysis in one or two bacterial systems [1], but frequently structures must be inferred from optical data (e. g., [2]). The complex with the simplest biological function is the "antenna," an array of rather similar chlorophylls which simply acts to increase the optical cross section of the reaction center (RC), the ultimate destination of excitation energy. Absorbed energy is channeled to the RC by excitation diffusion, i. e., the Förster resonance transfer process [3]. Inability to crystallize most CPs stems from their insolubility in water; this is characteristic of all green plant CPs, for example.

The Rochester group has examined the Thornber [4] antenna CP extracted from a variety of green plant species by detergent solubilization and has postulated a model of its chomophoric arrangement [2,5]. Aside from the fact that the protein characterized in this manner seems to be universal, a fundamental interest attaches to the study of these small systems. They are examples of an ensemble of complicated but nonetheless identical N-body systems where N is small (of the order of 6 to 100). The protein studied at Rochester has N=6 and displays both strong- and weak-coupling exciton states. It has interesting high-excitation-intensity fluorescence behavior [6,7] which enables exciton annihilation to be studied in a very small system.

Aggregates of CPs, both artificial and *in vivo*, have been subjected to 30-ps pulse excitation to examine their time-dependent fluorescent response [6,8]. Excitation annihilation, when analyzed in the context of the many other nonlinear effects at high excitation intensities, proves a capable tool for tracing excitation pathways. The Rochester

experimental work is based on use of a very low-jitter streak camera [9] and image intensifier enabling 2-ps timing accuracy. Despite using the 30-ps pulses, rise-time delays of the order of 5 to 20 ps can be obtained with good accuracy. Similar data, leading to new dynamical insights, have been achieved in studies on other biosystems such as tryptophan [10], DNA [11], and hematoporphyrin derivative [12].

Research supported in part by American Cancer Society grant IN-89H (Nordlund), National Science Foundation grants PCM-80-18488 (Knox and Mourou), PCM-83-02601 (Nordlund), and PCM-83-03004 (Knox), and U. S. Department of Agriculture grant 82-CRCR-1-1128 (Knox).

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SUMMARY OF THE PRESENTATIONS AT THE ASPEN WORKSHOP ON MEMBRANE BIOPHYSICS

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We are currently investigating the detailed characteristics of the single channel currents activated by cholinergic agonists from *Torpedo californica* acetylcholine receptors reconstituted in lipid bilayers. The two major goals under investigation are: 1) a kinetic scheme that will describe the relationship between cholinergic ligand binding and channel open states; and 2) to establish the functional specificity of individual acetylcholine receptor subunits in channel function.

Concerning the first objective, we described experiments that established that the probability of channel opening increased with cholinergic agonist concentration. The analysis of the probability distribution of open state lifetimes indicates that there are two distinct channel open states, short and long-lived. The frequency of occurence of the long openings over the short, increased with acetylcholine concentration, while the corresponding time constants of the short and the long-lived open states were unaffected. These results are consistent with the notion that the two open channel states arise from different extents of ligand occupancy at the receptor molecule. Furthermore, they are inconsistent with schemes that require a transition pathway communicating the singly-liganded state with a doubly-liganded state, since they imply that the time constants of the two open states would depend on agonist concentration. Focusing only on the open states scheme, a minimum kinetic scheme that accounts for the results is the following:

$$\begin{array}{ccccc} C + L & \rightleftarrows & CL + L & \rightleftarrows & CL_2 \\ & & \uparrow\downarrow & & \uparrow\downarrow \\ & & OL(\textit{short opening}) & & OL_2(\textit{long opening}) \end{array}$$

where C and O denote closed and open states of the channel and L is the ligand.

With regards to the second objective, we described experiments in which monoclonal antibodies directed against specific acetylcholine receptor subunits, are used as specific probes of acetylcholine receptor function. Monoclonal antibodies which bind to the main immunogenic region on the extracellular domain of the α subunits do not affect the single channel conductance or the channel lifetimes. In contrast, we have found a set of monoclonal antibodies that bind to the β and to the γ subunits and inhibit single channel activity. Thus, at the present time, it appears that the β and the γ subunits or their interactions with their neighbor subunits are critical for channel gating. We anticipate that this approach may allow a molecular mapping of the structure-function relationship in the acetylcholine receptor channel.

We also discussed our investigations on the molecular mechanism of tetanus toxin action. We have studied the interaction of tetanus toxin with gangliosides in planar lipid bilayer membranes. We find that the interaction of the toxin with the gangliosides results in the formation of transmembrane ion channels. The tetanus toxin channel has a single open state and two distinct closed states. The tetanus toxin channel is cation selective, and it is voltage dependent such that it activates as the compartment containing the toxin becomes progressively more positive. We described our inferences on the molecular mechanism of tetanus toxin action in situ from these observations in our model system. We suggested a scheme in which the insertion of the tetanus toxin channel in the postsynaptic membrane would perturb synaptic function by causing synaptic disinhibition. Thus, the activity of the tetanus toxin channel at post-synaptic membranes would lead to persistent activation via continuous depolarization, and therefore, would lead to spasticity and convulsions which are characteristic of human tetanus.

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Description of Research Discussed at
Aspen Center for Physics
Summer of 1984
John C. Owicki

During my stay at Aspen this summer I gave talks on two areas of current research in my lab in the Biophysics Dept. at U.C. Berkeley. I will summarize each below.

I. Interactions between Membrane Proteins. Biological membranes display many specific intermolecular interactions and lateral structural specializations. We have developed a method that allows fundamental analysis of the interactions between membrane proteins and may prove useful in relating these interactions to membrane structure and function (1).

Biological membranes and lipid bilayers are in many respects like two-dimensional fluids. Freeze-fracture electron microscopy is capable of revealing samples of the essentially instantaneous positions of intrinsic membrane proteins. The lateral distribution of the proteins is governed by intermolecular interaction potentials, and we have devised a method of deducing these potentials from positional correlations of the proteins that are revealed in the electron micrographs. The technique involves the solution of the Born-Green-Yvon integral equation from the statistical-mechanical theory of liquids.

We have applied the technique to gap junctions, regions of densely packed channel proteins that frequently bridge the extracellular gap between closely apposed cell membranes and allow small molecules to be shared between the cells. We found the protein-protein interaction to be repulsive and have interpreted the dense packing of the proteins in terms of a minimization of the repulsive energy between the closely apposed cell membranes in the junctional region. In addition to being the first ab initio analysis of interactions between membrane proteins, this work explains several features of the structure and formation of gap junctions.

II. Immunological Recognition in Model Biological Membranes. Many of the most important effector and regulatory functions in the immune system depend on noncovalent ligand-receptor recognition processes at membrane surfaces. We are studying the fundamental chemical and physical properties of such phenomena by analyzing the interactions of monoclonal antibodies with fluorescent haptens (antigens) that have been linked to the surfaces of phospholipid bilayer vesicles (2). The behavior of this simplified model system should illuminate the more complex events in real biological membranes.

We measure the antibody-hapten binding by monitoring the fluorescence quenching that accompanies the process. In an initial characterization of the system we discovered that bivalent

IgG antibodies bind the membrane surfaces more strongly than do monovalent antibody fragments, presumptive evidence that the IgG is bivalently attached to the membranes. This is of great significance for future biological applications of the system.

We have also discovered that the kinetic and equilibrium properties of the binding depend sensitively on the phospholipid composition of the target membrane. These observations, plus a variety of spectroscopic results (3) have led us to hypothesize that the hapten exists in an equilibrium between conformations extended out from the membrane (available for antibody binding) and sequestered at or in the membrane surface (not available for binding). Such crypticity appears to be a widespread property of model membranes and has analogs in the immunological reactivity of biological membranes.

Future investigations will aim at exploiting this well characterized system in studies on the mechanisms of membrane adhesion and the mechanism of the activation of serum complement by antigenic membranes.

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Research discussed by C. F. Stevens at the workshop concerned the problem of electrical excitability of nerves. Metabolic energy is used by all cells to concentrate potassium ions within the cell, and to pump out sodium ions. The result is that intracellular potassium concentrations are an order of magnitude greater than extracellular ones, and extracellular sodium is an order of magnitude more concentrated than intracellular. Calcium, chloride and hydrogen ions also have different inside and outside concentrations. Special membrane proteins, known as channels, make use of these ionic concentration gradients to produce electrical activity by permitting, in a highly regulated way, transmembrane flows of sodium and potassium ion currents. Different channel proteins control the flow of the various ions. For example, the sodium channel senses the voltage gradient across the nerve cell membrane and opens and closes a pore to control the sodium ion flux into the cell.

Discussions at Aspen centered on the sodium channel, the integral membrane protein primarily responsible for the nerve impulse. Techniques developed in recent years make it possible to measure the current controlled by a single sodium channel. This method, known as single channel recording, reveals that a current of about 1pA flows when a sodium channel opens and that the channel remains open for and average of about .5 ms before closing. Open times are random and exponentially distributed.

How do sodium channels open and close, and how is their behavior coupled to the transmembrane electric field? Protein molecules generally change their shape when they function, and such conformational changes are

responsible for opening and closing of sodium channels. This protein, unlike most, has a very large dipole moment that changes (more accurately, the component of the dipole moment normal to the membrane changes) when the protein switches from one conformation to another. Interactions of the membrane electric field (whose magnitude is about 100 kV/cm) with the sodium channel dipole cause certain conformational states to be energetically favored. Alterations in the electric field thus drive the sodium channel from one conformation to another, at least statistically.

Unfortunately, channels do not exhibit properties that are as simple as one might hope. Specifically, the sodium channel posesses three operationally defined classes of conformational states: these classes are resting, open, and inactivated. Channels normally reside in the resting state, but are driven into the open state when the voltage difference across the membrane is made more inside positive. Positive going voltage changes also cause channels to enter the inactivated state, and they cannot escape from this state until the voltage has been returned to the normal inside negative condition for a period of several milliseconds. There is the additional complication that at least some of these classes of states possess substates. To understand sodium channel operation, then, one must define the states that can be occupied and discover the rates of transition between them; additionally, a physical theory must be developed to account for these transition rates, and the kinetically defined states must be identified with specific protein structures.

The initial stages of this program involve analysis of the statistical behavior of the individual channel. The occupancy of the open state is directly measured, the occupancy of the inactivated state is estimated through a testing procedure that reveals how many channels have not yet become inactivated, and the occupancy of the adjacent-to-open state is found, up to a multiplicative constant, from the rate of entry into the open state. A "remainder" state is defined as the one that is not open, not inactivated, and not adjacent-to-open. Analysis of the channel behavior reveals that the equations implicit in this formulation provide an adequate description. Transition rates thus determined vary with voltage, and the next analytic step involves producing a theory for this voltage dependence. Outlines for such a theory are already evident, and it seems that the presence of fixed dipoles associated with each state account for the rates of conformational changes that occur when the membrane electrical field is altered. Continuation of this analysis should yield an understanding of the biophysics of neuronal electrical activity.

ABSTRACT

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The acetylcholine receptor is composed of five subunits with homologous sequences. The sequence analysis at the cDNA level suggests extensive homology at the amino acid level and alignment of these sequences shows that they are all homologous throughout their entire length. Periodic change in hydrophobic character of the amino acids within the sequence suggests that there is a region of alpha helical periodicity which strongly suggests a mechanism for ion channel formation between the five subunits. A model was generated based upon these five homologous sequences; this model explains many of the properties found by electrophysiological analysis for transduction of ions by the acetylcholine receptor. Consistency between this model and protein chemistry on the one hand, electro-physiology on the other, is examined.

Subunits within the acetylcholine receptor have been mapped using immuno-electron microscopy which shows that the two alpha subunits are separated by one other subunit. This is most probably the beta subunit as suggested by cross-linking evidence. Thus the arrangement of chains within and around the ion channel is suggested by these results.

Crystals of the acetylcholine receptor have been obtained and used to calculate an imaging construction of the structure within these crystals. It shows a five-fold nature characteristic of the expected pseudo-five-fold character of the receptor.

A proposed mechanism for channel opening and channel gating relies upon extensive conformation change between the closed state and the activated state. These states are examined by chemical, spectroscopic, and ultimately will be examined by genetic means.

RHODOPSIN, TRANSDUCIN, AND THE CYCLIC GMP PHOSPHODIESTERASE

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The photoexcitation of rhodopsin triggers a cascade that results in the hydrolysis of a large number of molecules of cyclic GMP. The molecular mechanism of this amplification cascade has been delineated. Transducin, a multisubunit peripheral membrane protein, is the information-carrying intermediate in the activation of the cyclic GMP phosphodiesterase. Photoexcited rhodopsin (R^*) catalyzes the exchange of GTP for GDP bound to the α-subunit of transducin (T). About 500 molecules of T_α-GTP are formed per absorbed photon at low light levels. T_α-GTP, released from the β and γ subunits of transducin, then activates the phosphodiesterase by relieving an inhibitory constraint imposed by its small subunit. Each activated phosphodiesterase molecule hydrolyzes more than a thousand cyclic GMP per second, giving an overall gain of about 500,000. Photoexcited rhodopsin triggers the activation of a molecule of transducin in a millisecond, which is sufficiently rapid to enable this cascade to participate in visual excitation. Hydrolysis of GTP bound to T_α serves to restore the sysem to the dark state. Transducin, like the G proteins of the adenylate cyclase cascade, can be specifically ADP-ribosylated by cholera toxin and pertussis toxins. In both cascades, labeling by pertussis toxin blocks the capacity of transducin to interact with the excited receptor, whereas labeling by cholera toxin inhibits the hydrolysis of bound GTP, leading to persistent activation. Moreover, the molecular design of the hormone-triggered cyclic AMP cascade is similar to that of the light-triggered cyclic GMP cascade. It seems likely that transducin, the stimulatory G protein, the inhibitory G protein, and the

ras protein are members of the same family of signal amplifiers. The study of the cyclic nucleotide cascade of vision is providing a view of a recurring motif of signal transmission and amplification in nature.

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SPECTRAL, TEMPORAL AND INTENSITY CHARACTERIZATION OF THE OPTICAL PROPERTIES OF SUSPENSIONS OF AGGREGATES OF PHYCOBILIPROTEINS ISOLATED FROM PHOTOSYNTHETIC MEMBRANES OF NOSTOC SP. USING PICOSECOND LASER SPECTROSCOPY

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Photosynthetic organisms have evolved a number of light harvesting antenna systems for the primary purpose of absorbing sunlight and transferring the absorbed energy to reaction centers where chemical oxidation and reduction occurs. In red and blue-green algae, the light harvesting system consists of aggregation of phycobiliproteins, namely phycoerythrin, phycocyanin, and allophycocyanin, which collectively form the phycobilisomes. Each phycobiliprotein chain is composed of two dissimilar polypeptide chains, the and subunits to which chromophores are covalently bonded. The number and the chemical nature of the chromophores depend on the origin and spectroscopic class of the phycobiliproteins. For Nostoc sp. the α and β subunits of phycoerythrin have two and four chromophores, respectively. The α and β subunits assemble into aggregates. We have studied the optical properties (quantum yield of fluorescence and transmission as a function of single pulse picosecond laser intensity, fluorescence kinetics and spectral dependence) of $\alpha\beta$, $(\alpha\beta)_3$, $(\alpha\beta)_6$, and $(\alpha\beta)_{12}$ aggregates in addition to aqueous suspensions of α and β subunits. The larger aggregates serve as useful model systems for probing non-linear, high intensity effects in small biological systems.

Our experimental results can be summarized as follows: (a) For α, β, and $(\alpha\beta)$ complexes the fluorescence decay profiles were found to be intensity independent for the intensity range investigated ($4x10^{13}$ to $4x10^{15}$ photons-cm^{-2} per pulse) (1,2). For larger aggregates of α and β the fluorescence decay times were shorten as the laser intensity increased. (b) α, β and $(\alpha\beta)$ exhibited an apparent decrease in the relative fluorescence quantum yield and an increase of the relative transmission with increasing excitation intensity. (c) For $(\alpha\beta)_3$, $(\alpha\beta)_6$, and $(\alpha\beta)_{12}$ the relative transmission was independent of laser intensity for the intensity range investigated although the relative fluorescence quantum yield decreased with increasing intensity (3). These differences in the quantum yield and transmission for small and large domains are shown in Figures 1-3.

The apparent fluorescence quantum yield dependence on excitation intensity has a functional form similar to that predicted by excition annihilation theories (4,5), even though these theories do not include the non-linear optical effects produced by ground state depletion or upper excited state absorption. Current exciton annihilation theories are known to be applicable only in cases where transmission is unchanged over the intensity domain measured (5). Further evidence against excition annihilation occurring in α, β and $(\alpha\beta)$ systems is that the emission decay, although non-exponential is intensity independent. The observation that the relative transmission (T) and fluorescence yield (ϕ) are approximately mirror reflections of each other is strongly suggestive of ground state depletion since for a three level model, under steady state excitation, $\phi = T^{-1}$ when the excitation intensity is less than the saturation intensity. Our results strongly suggest that annihilation processes do not occur in isolated α, β and $(\alpha\beta)$ units for fluences less than $4x10^{15}$ photon-cm^{-2}. In the case of aggregates $(\alpha\beta)_3$ and larger the experimental results

in Figures 2 and 3 indicate that the size of the complexes are sufficiently large enough for more than two excited states to exist simultaneous, i.e., excition annihilation does occur. An analysis of the temporal decay kinetics supports the notion that both "s" and "f" chromophores absorb and fluoresce. The experimental data can be accounted for quantitatively in terms energy transfer between "s" and "f" moieties. The Förster distance (Ro) and the distance between the s and f moieties calculated using polarization, spectral and temporal fluorescence data are consistent with known molecular dimensions of the phycobilisomes (6).

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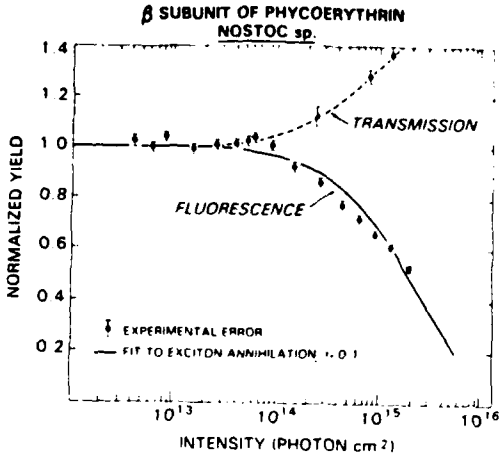

Fig. 1. (a) Apparent quantum yield of fluorescence and relative transmission as function of laser single pulse intensity (photon -cm^{-2}). Solid line is fit of apparent relative fluorescence quantum yield to the Paillotin-Swenberg theory with r=0.1. This fit as discussed in text in facillous.

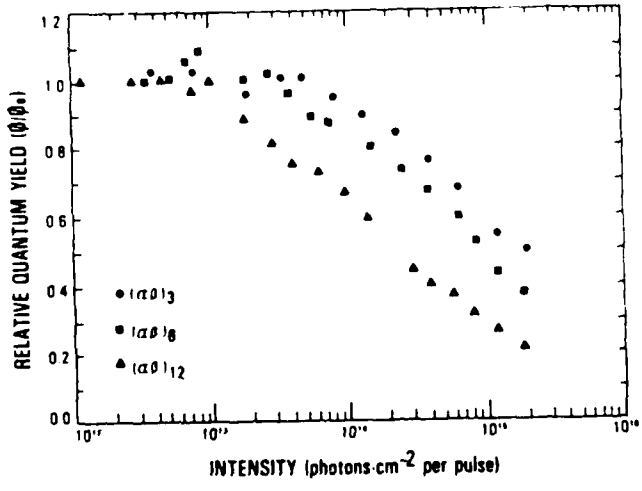

Fig. 2. Fluorescence for the $(\alpha\beta)_3$, $(\alpha\beta)_6$ and $(\alpha\beta)_{12}$ aggregates.

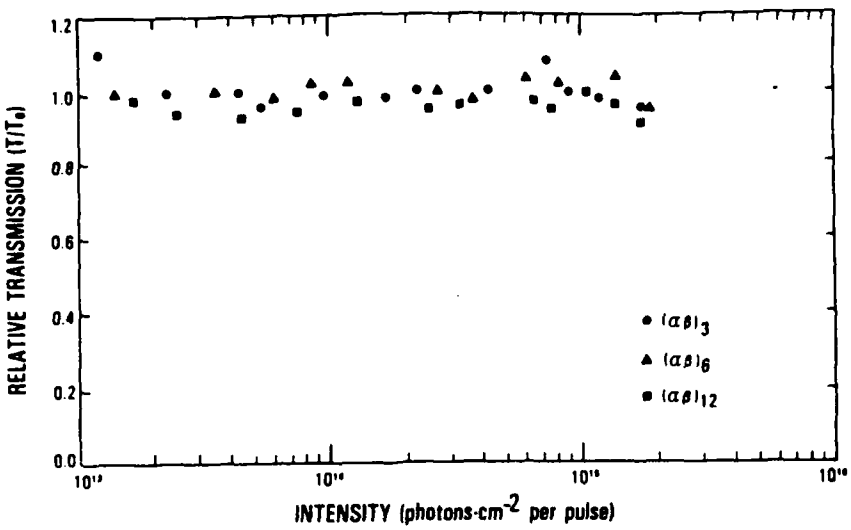

Fig. 3. Relative Transmission as a function of laser pulse intensity.

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